REGENERATION IN A TROPICAL EARTHWORM *PERIONYX EXCAVATUS* E. PERR.

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In the course of a recent study of the *Oligochæta* of Rangoon a number of earthworms were found with reproductive apertures in normal arrangement and position on the segment but displaced one to four metameres anterior to or one to three metameres posterior to their usual position. The prostates and ovaries had the same definite relations to the sexual orifices in such individuals as in normal specimens and hence were anterior or posterior to their ordinary position to an amount equivalent to the dislocation of the reproductive pores. None of the various metameric and organ anomalies that appear in nearly all of the species of Rangoon earthworms were noticed in specimens of the type just mentioned.

Morgan (1895) found similar anomalies in specimens of *Allolobophora fœtida* and suggested (p. 404) that "In those cases in which openings of the vasa deferentia occur on a segment anterior to the 15th metamere,[1] we may be dealing with a case of incomplete regeneration of the anterior metameres. . . ." But further on he says, "That all of the cases can be explained in this way is, I think, highly improbable," and (p. 450) "regeneration of the anterior end will not account for any of those cases where the vasa deferentia open on a matemere posterior to the fifteenth."

No references to hypermeric regeneration of anterior ends in earthworms could be found in the literature available. The abnormality (posterior dislocation) was found so frequently, however, that a series of operations was begun with the idea of learning whether or not such anomalous specimens would develop as the result of regenerative processes experimentally induced in earthworms of the species concerned. One of the animals first used seemed to have such an unusual capacity for replacing lost parts that an extensive series of experiments was initiated to discover the limits of this capacity. After several months it became

[1] In *A. fœtida* the normal position of the male pores is on segment fifteen.

necessary to discontinue the work. Some of the data accumulated are of such interest, however, as to warrant publication in a preliminary note. At the first opportunity the experiments will be resumed.

In the preliminary series of operations worms belonging to nearly all of the species occurring in Rangoon were anæsthetized and the anterior ends excised at various levels. Only individuals of two species, *Pontoscolex corethrurus,* and *Perionyx excavatus* survived more than five days after the operation. At the end of two months only a small amount of segmentally undifferentiated tissue had been produced at the cut ends of worms of the first species, whereas all operated specimens of the second species had regenerated, in much shorter time, segmentally differentiated anterior ends. Further experiments were confined to this second worm.

Adult specimens of *P. excavatus* attain a length of 130 mm., and a diameter of 5 mm. The prostomium is large, fleshy, and characteristic. The intersegmental furrows are deep and clearly marked. Secondary annulation is lacking. The dorsal and lateral parietes are heavily pigmented. The clitellum is ring-shaped, yellowish or gray, contrasting sharply in color with the non-clitellar segments, and lies between the intersegmental furrows 12/13 and 17/18. The setæ are numerous and arranged in a closed ring around each segment behind the first metamere. The spermathecal apertures are large paired pores in the intersegmental furrows 7/8 and 8/9 (paired spermathecæ in viii and ix). The single female pore is clearly visible on xiv (paired ovaries in xiii). The larger male apertures are closely approximated mid-ventrally on xviii (paired prostates in xviii, paired seminal vesicles in xi and xii, paired naked testes in x and xi).

Mature worms can be secured in large numbers all the year round. They easily adjust themselves to the conditions of life in the laboratory, and have an exceedingly low mortality rate after operation or injury. The sharply delimited clitellum and large sexual apertures enable rapid determination of position, while the clear cut intersegmental furrows, the absence of secondary annulation, and the distinctly projecting setal circles, render segment counting simple, and the detection of metameric and setal anoma-

lies easy. The dark red color of the normal segments sharply sets off the much lighter colored new tissue during the early weeks of regeneration and the presence of a protruding fleshy prostomium distinguishes a regenerating head from any other structure.

Only fully mature and normal worms were used. They were anæsthetized in a weak solution of chloretone and an excision was made by a clean cut, usually at an intersegmental furrow. The operated worms were kept in closed jars containing moist paper towelling. All results described in this paper were obtained in that part of the year known locally as the "cold season." The term is of course merely relative and indicates that the mercury is slightly lower all day long than at other seasons of the year. No effort was made to control the temperature but in the brick laboratory building the fluctuations of the mercury from midday to midnight are much less marked at this season of the year than out of doors.

In a short time after the operation a transparent conical outgrowth was visible at the cut end. In the case of regenerating anterior ends, oval fæcal pellets were found from the sixth day on, indicating that the digestive system had developed sufficiently in that short time to enable the worm to "bite" off, swallow, pass through the digestive system, and defecate pieces of the paper towelling. By the end of the second week the segmental differentiation of the regenerating heads was completed. Usually by the end of the third week the new segments contiguous to the old tail piece had attained the diameter of the metameres with which they were in contact, and were clearly setigerous. In the fourth week the pigment appeared in the regenerating segments.

Regeneration of Anterior Ends.

In all series of operations every surviving worm from which six or fewer segments had been amputated regenerated the exact number lost. If the prostomium or a fraction of the prostomium was removed a new prostomium or the missing fraction was replaced. In several worms a small wedge-shaped piece was removed dorsally, laterally, or ventrally from the anterior end of the stump. In each case such wedge-shaped pieces were replaced as well as the exact number of missing anterior segments. Only

one animal died following operation anterior to segment six and in this single instance there was reason for suspecting that the death of the worm was not caused by the effect of the operation on the individual but by another factor to be discussed later.

When the excision was made at an intersegmental furrow posterior to 6/7 but anterior to 18/19 the ratio of the number of segments removed to the number regenerated, varied and the percentage of failure to survive the operation was higher. Here again there was reason for suspecting that many of the mortalities following operation were due to something more than the effect of the operation itself. None of the amputations at intersegmental furrows 10/11 or 14/15 resulted in hypomeric regeneration, but the number of worms involved (five at the first furrow and two at the second level) is too small to permit generalization. At all other intersegmental furrows between 6/7 and 18/19 hypomeric anterior ends have been formed. The largest number of segments not replaced is four and the smallest is one.

In one series four worms regenerated hypomeric heads each having one "half segment," *i.e.*, a metamere extending across the whole diameter of the animal but antero-posteriorly only about half the width of the segments immediately in front of and behind it. Such half segments were not setigerous and may represent incompletely differentiated metameres, or perhaps growth zones, although the specimens concerned were killed in the fourth week after the operation, by which time the segmental differentiation is usually completed. In only one regenerating anterior end was a wedge-shaped half-segment found and this was ventral in position.

Excisions at levels 7/8, 9/10, 10/11, and 13/14 alone resulted in hypermeric regeneration but the total number of such cases is too small for generalization. Only one extra segment was formed in each case. In these hypermeric worms all the regenerated segments except of course the first were setigerous and clearly outlined by intersegmental furrows. No half-segments, wedge-shaped or otherwise were found in heads of this type.

When the anterior end was excised posterior to 17/18 only hypomeric heads of ten to sixteen segments were formed. The number of worms operated on behind this level was too small to

warrant a statement that total replacement of lost segments cannot take place posteriorly. It should be noted that when eighteen or more segments were amputated the digestive organs including part of the intestine which begins in the region of segment fifteen, as well as all of the reproductive organs were removed. When the cut was made between 17/18 and 24/25 twelve to sixteen segments usually regenerated. Posterior to 24/25 ten to fifteen segments regenerated. The posterior limit of anterior regeneration by tail pieces has not been determined, but it certainly lies unusually far back for earthworms, and at least in the last third of the length of the worm.

TABLE I.

SERIES TWO.

Anterior Ends from One to Twenty Segments Removed.

85 worms were amputated.
81 worms survived operation.
48 worms regenerated exact number of segments lost.
27 worms regenerated a smaller number.
5 worms regenerated a larger number.
1 worm failed to regenerate.

Table I. summarizes briefly the results obtained from a characteristic series of operations. Table II. summarizes the regeneration in the region lying between segments six and eighteen

TABLE II.

SERIES TWO.

Results of Operations between Intersegmental Furrows 5/6 and 17/18.

Number of Segments Removed.	Number of Specimens Operated.	Worms with Regeneration of Exact Number of Segments Lost.	Worms with Hypomeric Heads.	Worms with Hypermeric Heads.	Worms without Regeneration.
7	7	4	2	1	—
8	8	5	3	—	—
9	10	5	3	2	—
10	5	4	—	1	—
11	2	1	1	—	—
12	5	1	3	—	1
13	4	2	1	1	—
14	2	2	—	—	—
15	4	—	4	—	—
16	3	2	1	—	—
17	4	2	2	—	—

from the second series of operations. In Table III. is a condensed statement of hypomeric and hypermeric regeneration in the same region but including results of more than one series of operations.

Table III.

Hypomeric and Hypermeric Anterior Ends Regenerated at Cuts between Segments Six and Eighteen.

Number of Segments Removed.	Number of Segments Regenerated.
7	3, 6, 8
8	6, 7
9	6, 8, 10
10	11
11	8, 10
12	8, 10
13	11, 14
14	— —
15	11, 12, 13
16	14
17	13, 14, 15

Regeneration of Posterior Ends.

No special attempt was made to study tail regeneration by anterior pieces. Numbers of amputated anterior portions were kept in conditions similar to those of the regenerating tail pieces and the daily records of the experiments contain some notes on these amputated pieces. Anterior ends of more than twenty segments may regenerate new tails. No information is available as to tail regeneration anterior to segment twenty. Amputation at various levels posterior to 20/21 usually resulted in rapid formation of new posterior ends. None of these regenerating anterior portions were kept alive longer than four weeks so that it is not possible to make any positive statement as to the ratio of segments replaced to those lost. There is no reason to suspect, however, that anterior portions of twenty segments or more cannot replace all segments especially if provided with food.

Mutilations.

While the first series of experiments was under way a collection of worms containing several regenerating specimens of *P. excavatus* was brought into the laboratory. Arrangements were

at once made to secure several thousand worms of this species from various quarters of the town. A considerable number of individuals thus obtained had evidently been deprived in some manner of a head or tail or quite rarely of both ends. One collection of more than three hundred worms contained more than a hundred mutilated specimens. This percentage was so high that previous digging was suspected of being the cause of the mutilations. As the collections had been made on successive days over a period of several weeks, it is possible that some at least of the mutilated specimens were produced in this way. In order to avoid this factor, collections were made at several localities which presented every appearance of having been undisturbed for months. Such collections also contained high percentages of mutilated individuals. Practically all the mutilations found were amputations of a head or a tail at an intersegmental furrow. Only three regenerating individuals were found in which excision had occurred in the middle of a segment. In these specimens the missing half segment had been regenerated as well as a portion of the tail behind. One worm mentioned elsewhere had been deprived of dorsal portions of two segments in addition to the anterior end.

Table IV. summarizes the information available from records of the collections. It should be noted that all mutilations included within this table had been produced at least several days previous to the time of collection. Such few specimens as were brought into the laboratory obviously injured as the result of collecting processes were, of course, discarded and not included in the tables.

Through such collections more than fifteen worms were secured that had lost their heads. Nine were regenerating new anterior ends when brought into the laboratory. Of this latter number four were either immature or if mature had lost more than eighteen segments, for there were no characteristic sexual markings to make a determination possible. The remaining five specimens had lost their heads anterior to the prostatic segment. By assuming that the prostatic segment of each of these animals was the eighteenth metamere before the mutilation, as in normal worms, it was possible to determine the number of segments lost and the type of regeneration that ensued. On the basis of this assump-

tion three individuals had regenerated hypomeric anterior ends, one had produced a hypermeric anterior end and the others had exactly replaced the number of segments lost. The single worm with hypermeric regeneration had lost the first thirteen segments as well as dorsal portions of segments xiv and xv and had not only replaced the lost dorsal portions but had also formed *fifteen* perfectly normal and clearly outlined setigerous segments and the non-setigerous prostomial segment.

Table IV.

Collection Number 1.

Normal worms	231
Mutilated worms	104
Worms with tail mutilation	98
Worms with head mutilation	6
Worms regenerating a tail	77
Worms regenerating a head	3

Collection Number 2.

Normal worms	301
Mutilated worms	87
Worms with tail mutilation	84
Worms with head mutilation	3
Worms regenerating a tail	71
Worms regenerating a head	2

Collection Number 3.

Normal worms	1
Mutilated worms	6
Worms with tail mutilation	5
Worms with head mutilation	1
Worms regenerating a tail	3
Worms regenerating a head	1

Collection Number 4.

Normal worms	?
Mutilated worms	?
Worms regenerating a tail	49
Worms regenerating a head	4

It is evident therefore that both in its natural environment and under experimental conditions in the laboratory *P. excavatus* may regenerate hypermeric anterior ends. In view of this demonstration there seems to be no need for further search, at least for the present, for other explanation of the origin of the anomalous

specimens mentioned at the outset of this paper. Such abnormalities at least in the species under discussion appear to be adequately accounted for as the products of hypo- or hyper-meric regeneration.

Varia.

Additional extensive experiments were begun to obtain information as to the posterior limit of anterior regeneration by tail portions, the anterior limit of posterior regeneration by head portions, the regeneration of pieces with two-cut ends from various regions of the body, etc. These experiments failed completely, as far as the objects in view were concerned, because of a constant series of accidents which will be described under the title of autotomy. A few notes from the records of these experiments are given herewith to indicate more clearly the unusual regenerative characteristics of this worm.

A. A short piece of eight segments from the middle portion of worm 109 was still living on the eighth day after the operation and responded quickly to various sorts of stimuli. Both ends had healed over without any signs of regeneration.

B. The forty-one anterior segments of worm 110 regenerated in nine days new tissue five and one half millimeters in length.[1] and fixing.

The new tail was composed of a long, metamerically differentiated portion containing more than thirty segments, the anal segment, and between these two a short region of formation of new metameres.

C. The anterior end of worm 132, a piece fifteen millimeters in length, composed of twenty-four segments regenerated in two weeks a tail fifteen millimeters long containing in the segmentally differentiated region, fifty-four segments.

D. A shorter anterior end from another worm regenerated at its posterior cut surface a *head* of several segments with a characteristic mouth and prostomium.

E. A twenty-three metamere fragment thirteen millimeters in length from the posterior half of worm 118 in two weeks regen-

[1] The measurements noted were made on material that had been killed by dropping into strong methylated spirits and then hardened in formalin. Old and new tissue appeared to be uniformly contracted by this mode of killing

erated at one end a new head containing thirteen segments and a prostomium and at the other end a tail two and one-half millimeters long, containing in addition to the anal segment and the growing region twelve differentiated segments.

F. A shorter portion from the posterior half of another worm regenerated at one end a head and at the other end a single anal segment.

G. A short tail fragment regenerated at its injured anterior end a structure exactly similar in appearance to the tail developed at the cut posterior surface of an anterior piece.

H. The nine anterior segments containing both pairs of spermathecæ were removed from worm 82. Eight segments regenerated. Characteristic spermathecal pores appeared in intersegmental furrows 6/7 and 8/9 (the posterior pair of pores being located between the last of the old and the first of the new segments!).

I. Several other worms from which anterior ends containing one or both pairs of spermathecæ had been removed regenerated heads with one or two pairs of spermathecal pores at various intersegmental levels. These worms were killed three weeks after the operation, hardened in formalin and dissected. Definitive spermathecæ had not been formed by that time. The site of each spermathecal pore was marked internally by the presence of a lump of soft spongy tissue. Some of these specimens with regenerated spermathecal pores were very similar to specimens of this species secured by Beddard (1886) from the Philippines. It is quite possible if not probable that many or even all of the thirteen anomalies described and figured by Beddard as "variations" were the result of regenerative processes.

J. Seventeen anterior segments were removed from worm 149. When the animal was killed at the end of the fourth week after the operation, seventeen segments had been regenerated. In the usual position on segment fourteen was a typical female pore. The clitellar segments (xiii–xvii) were distinctly lighter in color than the other new segments, indicating the beginning of clitellar differentiation. Although the head was carefully fixed and hardened the tissues were too soft and spongy internally for dissection and no reproductive organs could be demonstrated.

Autotomy.

Several references have already been made to disturbing factors which interfered with the success of some of the experiments. One of factors, the most important, was a tendency for the worms to break into fragments in early hours after the operation. For want of a better term this process of fragmentation will be referred to as autotomy. Only very rarely was this fragmentation observed to occur later than the first twenty four hours after the operation, and then only very small portions usually consisting of one or two segments were thrown off.

In the first series of operations on *P. excavatus* many of the operated worms autotomized one or more pieces from the posterior portion. In another series of anterior operations thirty two out of forty two animals autotomized portions of the tail ranging from seven to sixty millimeters in length. No series of operations in which tail portions of the worm were watched was free from this tendency to fragment. In the majority of cases one or two short pieces were autotomized from the posterior end of the major operated portion. Such fragments were usually dead when discovered but very often lacked the pungent odor so characteristic of decaying earthworm. Occasionally the fragmentation was much more striking and extensive. Worm *B* 9 from which eleven anterior segments had been removed broke into pieces. Worm *D* 19 from which *X* anterior segments had been removed broke entirely into pieces six to ten millimeters in length. Several other specimens from which eight or nine segments had been removed also autotomized extensively.

Autotomy is usually understood to be a throwing off by the animal of a small portion which usually dies without producing a new animal but in *P. excavatus* apparently any fragment from any region may survive, or more than one of the fragments may survive, with the survival determined by the presence or absence of something in the worm and not by the position of the fragment along the axis of the animal. The autotomy was observed only in posterior portions. The length of the tail however was of no significance. Posterior portions from one third to approximately nine-tenths of the length of the original worm autotomized

extensively while anterior portions longer than one-half never autotomized. Several posterior portions about equal to one-half the length of the original worm autotomized from each end one or two pieces which died while the longer middle portion survived.

The experiments were discontinued before a thorough analysis of various aspects of this interesting tendency to fragment could be completed. Two experiments, however, provided a hint, not only as to the cause of the fragmentation but also as to the cause of certain other disturbing factors. Worm 94 was anæsthetized as usual and cut into three approximately equal portions, each of which was kept separately in a tightly closed jar. No autotomy occurred in any of the jars. The head piece survived and regenerated a tail. The middle piece regenerated at one end an anal segment and at the other end a head about three millimeters in length composed of fifteen segments. On the day following the operation the tail portion was collapsed and flattened, dead, but without noticeable odor of decay. In the jar were three flies which must have been present in the tail portion of the worm at the time of the operation, as the jar was not opened until after the flies had appeared. Through the kindness of entomologists at the Imperial Bureau of Entomology, London, these flies have been identified as *Aphiochæta scalaris* Lw.

A number of head portions ranging from twenty-five segments to about half the length of the worm were kept in a single, large, tightly-closed jar. A few portions died during the first three days after the operations and were removed. At the end of the week four of the head pieces were still living and apparently in good health although without signs of regeneration. The only traces to be found of the other head portions were numerous tubular fragments of transparent cuticle. Crawling around inside the jar were numerous small insect larvæ. When the jar was opened two small flies very similar in appearance to those from the other worm escaped. There seems to be no reason for doubting that some larval stage of the fly was parasitic in these worms at the time of the operation and it is at least possible that the presence of parasitic fly larvæ is the factor responsible for the autotomy as well as other disturbances in the operated worms. It should be noted, however, that *A. scalaris* is a very general feeder and that

it has been bred from all sorts of decaying matter. The Director of the Imperial Bureau of Entomology writes: "I should be inclined to suppose that the attack on the earthworms that you have noticed was accidental, for it seems unlikely that this fly would prove to be a true parasite."

Summary.

1. *P. excavatus,* an earthworm occurring in large numbers in dung heaps and soil rich in decomposing organic matter in Rangoon has a regenerative capacity very much higher than any known at present from megadrilous Oligochæta with the single exception of the limnic *Criodrilus lacuum* Hoffm., from Europe. The rate at which regeneration is completed is rapid.

2. Posterior portions can replace the anterior segments lost if the number of metameres removed is seventeen or less. When more than seventeen segments are removed only ten to fifteen metameres were regenerated.

3. The posterior limit of head regeneration lies somewhere in the last third of the length of the worm.

4. Spermathecal apertures and female reproductive pores may develop on regenerating anterior ends.

5. Anterior pieces of twenty segments or more may regenerate tails.

6. A heteromorphic head may be regenerated at the posterior end of a very short anterior piece.

7. A heteromorphic tail may be regenerated at the anterior end of a very short tail piece.

8. A piece of twenty or more segments from the middle of the worm may regenerate at one end a tail and at the other end a head.

9. Regenerated heads may be normal, hypomeric, or hypermeric. Hypomeric and hypermeric regeneration is considered an adequate explanation of the origin of abnormalities described as anterior or posterior dislocation of the reproductive organs.

10. In collections made in various quarters of the town a high percentage of the individuals secured had been mutilated by the amputation of a head, a tail, or both. Many of the mutilated specimens were regenerating the lost parts when collected.

11. One or more pieces both anteriorly and posteriorly are very frequently autotomized after operation by posterior portions. Sometimes the whole tail portion fragments into pieces six to ten millimeters in length. Anterior ends have not been observed to autotomize.

12. A fly *A. scalaris* Lw. has been bred from portions of *P. excavatus*. Parasitism by this insect may possibly be responsible for the mutilated specimens and for the autotomy following operation.

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